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Examination of Chemical Adsorption and Marine Biofouling on Metal Surfaces Using Raman Scattering Techniques and Electrochemical Impedance Spectroscopy (U)

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

A System has been developed to simulate biofouling of metal surfaces under a range of physical, chemical, and biological conditions. Items of equipment to eliminate and concentrate organic constituents from seawater and to analyze for proteins, glycoproteins, and carbohydrates have been acquired. Teflon fouling chambers have been developed to characterize surface properties of metals in seawater, i.e., adsorption of organic materials, using optical and electrochemical spectroscopic probes. Preliminary studies using these chambers and surface-enhanced Raman spectroscopy demonstrated ability to detect thin layers of pyridine, tryptophan, and phenylalanine adsorbed to silver electrodes. A system to perform waveguide Internal Reflectance Raman Spectroscopy (WIRRS) on thin films adsorbed to a substratum was developed and tested. During preliminary studies, an excellent Raman spectrum was obtained from a 1 μm layer of polystyrene. For Electrochemical Impedance Spectroscopy (EIS), electronic hardware
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was acquired and software was developed to determine the impedance attributes of fouled metal coupons. Preliminary EIS studies using the enzyme, Ribulose Biphosphate Carboxylase, demonstrated the ability to detect adsorption of this protein to a silver electrode.

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PRINCIPAL INVESTIGATOR: Gordon T. Taylor

CO-INVESTIGATORS: Shiv K. Sharma, Bruce E. Liebert & Howard F. Mower

CONTRACTOR: University of Hawaii at Manoa

CONTRACT TITLE: Examination of Chemical Adsorption and Marine Biofouling on Metal Surfaces using Raman Scattering Techniques and Electrochemical Impedance Spectroscopy

START DATE: 1 November 1987

RESEARCH OBJECTIVES:

Our primary objective is to describe the initial chemical and microbiological events which occur on virgin metal surfaces immersed in seawater using primarily non-invasive, in situ analytical techniques. To accomplish this, we've defined the following specific objectives.

(1) Develop optimal spectroscopic sampling strategies for in situ detection and identification of low concentrations of adsorbed organic materials, including spontaneous Raman Scattering (SRS), surface-enhanced Raman scattering (SERS), UV-vis Resonance Raman scattering (RRS), Waveguide Internal Reflectance Raman spectroscopy (WIRRS), Electrochemical Impedance spectroscopy (EIS), and fiber-optic probes.

(2) Obtain spectra and detection limits for putative fouling compounds; proteins, humic and fulvic acids, glycoproteins, polysaccharides, and fatty acids using purified compounds adsorbed to metals in organic-free seawater.

(3) In situ identification of primary adsorbing compounds on metal surfaces in a flowing seawater system in the absence and presence of planktonic microbial communities.

(4) Compare coupons fouled in the field (nearshore Oahu, Hawaii and open ocean) with those fouled in the laboratory.

(5) Quantify rates of chemical adsorption and microbial attachment to selected metal surfaces under varying nutrient, biomass, temperature, light, and flow regimes.

(6) Characterize spatial heterogeneity of adsorption and microbial attachment on individual coupons.

PROGRESS (YEAR 1):

Much of our first year has been dedicated to acquisition, engineering, and technique development. Although funds arrived late, we have received all major items of equipment, fabricated and tested several apparati, and have obtained SERS and EIS spectra for several materials adsorbed to metal.

We have developed an environmentally-controlled, flowing seawater chemostat system with test metal coupons in Teflon flow-through fouling chambers (Fig. 1). The system is designed so that the dilution rate, i.e., microbial growth rate, and the flow rate across the test coupons are controlled independently by two separate pumps. The system is well-oxygenated to prevent anaerobiosis in the bulk phase. The fouling chambers are designed such that there is minimal disturbance in the flow field, i.e., the test coupons are counter-sunk within the flow chamber. The optical flow chambers have 1.5 or 2" quartz windows which permit observation and passage of laser excitation and scattered radiation between the test coupon and the microscope objectives of the Raman spectrometer. The electrochemical cell uses the test coupon as a working electrode and has a matched platinum counter electrode in the upper plate, sealed leads to both electrodes, and a blind duct for the working solution (seawater) leading to a glass frit, electrolyte, and a reference electrode. A modified electrochemical cell with window has been developed in order to perform SERS analysis of adsorbed films (described below).

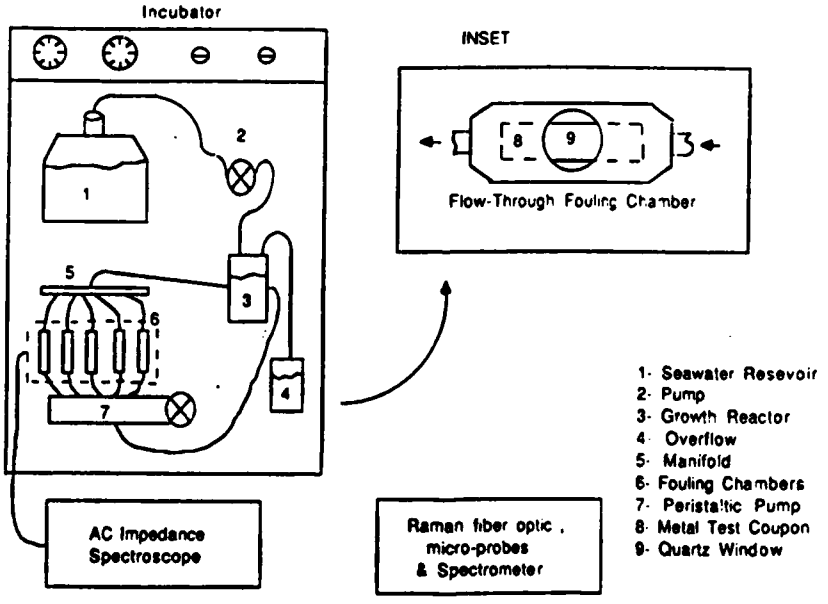

Figure 1. Schematic of continuous culture apparatus with multiple fouling chambers used to simulate and monitor marine biofouling.

We have acquired and developed systems for water collection,

filtration, isolation of molecular size fractions, and reduction of organic loadings for large volumes of seawater. For collection of uncontaminated seawater with minimal disruption of the plankton, an all polymer system comprised of a portable DC vacuum pump which evacuates a reservoir and draws water from 50 - 100' was developed. The system is easily operated from a small boat and can collect 100 liters in less than an hour. For filtration, we have acquired a 142 mm Teflon filtrator for particulate removal and an AMICON DC10L Hollow Fiber Concentrator/Dialyzer to remove and concentrate particles > 0.1 μm and molecular fractions; 100 kDa - 0.1 μm and 10 - 100 kDa. If desired, the 1 - 10 kDa molecular size fraction can be concentrated using our AMICON Ultrafiltration membrane apparatus. These molecular fraction concentrates will be used in fouling experiments and will be characterized chemically using Raman spectroscopy, FT-IR spectrometry, gel electrophorhesis, ion chromatography with PAD for carbohydrate and glycoprotein, and spectrophotometry. To remove residual organics from the seawater, we have acquired a two-stage activated carbon cartridge system which is the final step in our water treatment protocol. Efficacy of the entire system will be tested early in Year 2. We have acquired a DIONEX Pulsed Amperomteric Detector and the appropriate columns for an existing DIONEX Quaternary Gradient Ion Chromatograph for the purpose measuring carbohydrates and glycoproteins. We have also purchased a BIO-RAD Mini-Protean II Gel Electrophorhesis system for characterization of proteins. Using non-ONR funds, we have augmented our analytical capabilities by acquisition of a Perkin-Elmer Model 1720X FT-IR spectrophotometer, data station, and Multiple Internal Reflectance (MIR) attachment with Ge crystals. This permits inter-comparison of our samples with previous work from other labs. We will also soon have a Reflectance-Absorbance attachment for the FT-IR for the direct examination of metal surfaces. We have also obtained matching funds from the DoD and the University of Hawaii to purchase a BOMEM DA3 near-infrared FT-Raman spectrometer system with a 1.06-μm laser. Using a near-IR probe on our adsorbed films will be advantageous because excitation radiation in this spectral region produces fluorescence-free Raman spectra and produces much less thermal decomposition than visible radiation. We expect delivery of this system in March 1989.

As one approach for studying the interactions between dissolved biomolecules and metal surfaces in seawater, we elected to use Surface Enhanced Raman Spectroscopy, SERS. We designed a three electrode electrochemical cell comprised of a silver working electrode (1-mm wire insulated with epoxy or 1-cm^2 coupon), a platinum wire counter electrode, and a calomel electrode separated from the working solution by a capillary (Fig. 2). Teflon was selected as the supporting body for all fouling chambers because of its inert nature and low surface energy and a quartz glass window was fitted for spectrophotometric observation.

To gain experience, we tested and optimized our cell using a well-known analytical system (pyridine / 0.1N KCl). The working electrode's surface was prepared by polishing with successively finer grades of alumina down to 0.05 μm, then sonicated in deaerated, distilled water. Surface roughening was produced by a symmetric double potential step waveform Oxidation-Reduction Cycle (ORC). A potentiostat regulates the static potential within the cell and a coulometer integrates the current passed through the electrode. The electrochemical cell with polished electrode is stabilized at -0.2 V and stepped up to +0.2 V until 3.6 mCoulombs have passed through the electrode. After conditioning, spectra were acquired at a -0.6 V potential.

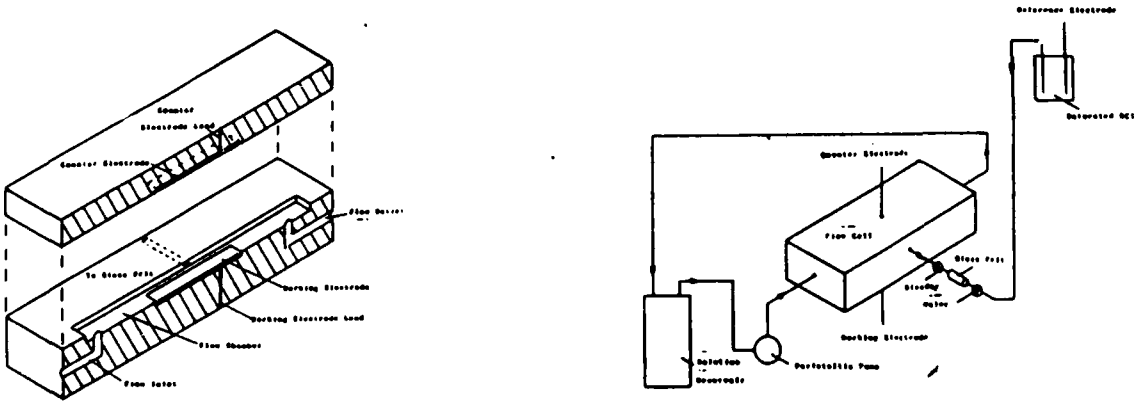

Figure 2. Schematic of flow-through SERS and EIS sampling systems. SERS cell has optical window over test coupon and offset platinum wire counter-electrode. For SERS, either flat metal coupon or wire can be used as the working electrode.

To determine how SERS would perform in a marine system, we examined 50mM pyridine in filtered UV-oxidized sea water as the electrolyte. On electrochemically-roughened silver, we obtained a 60-fold enhancement in Raman scattering intensity of the phenyl ring breathing mode (~ 1000 cm^{-1}) for pyridine over the bulk phase and 30-fold enhancement over unconditioned silver at its resting potential (Fig. 3). The lowest detectable signal was obtained using a 10^{-7} M pyridine solution.

Preliminary SERS studies on amino acids, peptides, and proteins are currently underway. Aromatic amino acids were chosen as target molecules because the amine group is known to interact with the silver surface and the phenyl ring is a good Raman scatterer. We have obtained SERS spectra of tryptophan and phenylalanine adsorbed onto a silver surface, but problems associated with fluorescence are particularly evident in the phenylalanine spectra (Fig. 4). Future studies will include more amino acids and synthetic peptides to gain more information on how protein primary structure interacts with the metal surface.

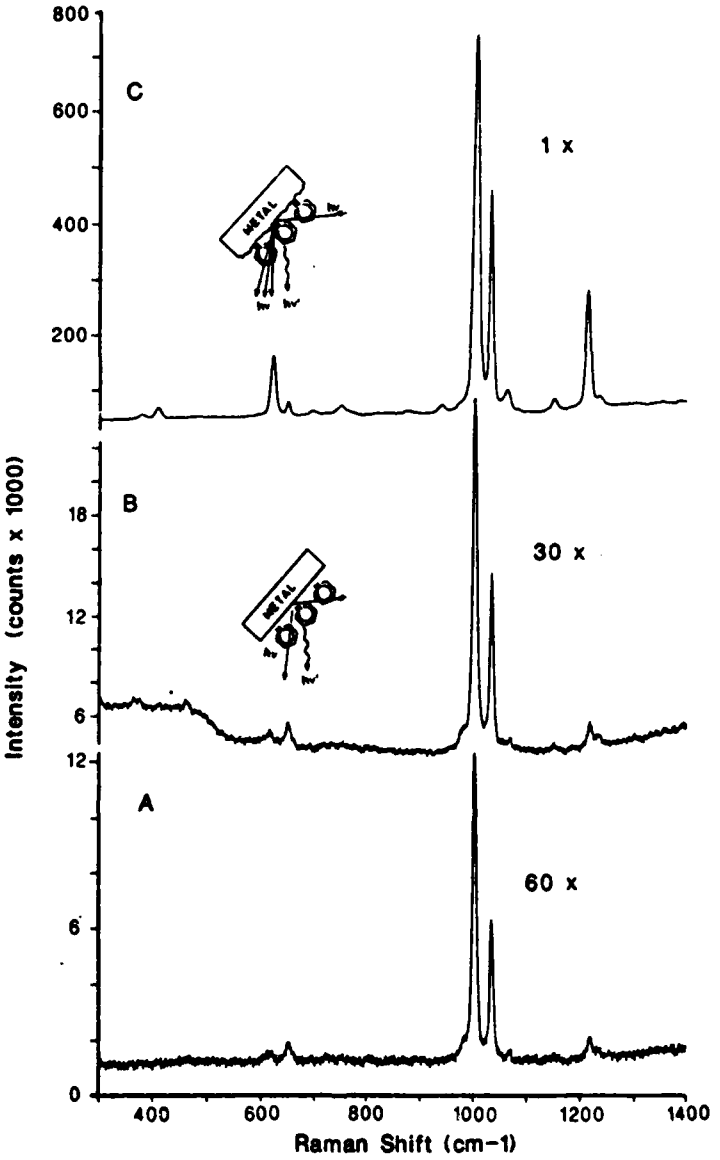

Figure 3. SERS study of pyridine. Excitation = 488.0 nm, 6 mW; 120 scans, 1 sec exposure. A. bulk pyridine (50 mM) in UV-oxidized seawater. B. pyridine adsorbed on polished Ag electrode in seawater, no ORC or applied potential. C. pyridine adsorbed to conditioned Ag electrode (30 cycles -0.6 to +0.2 V) and measured at a -0.6 V potential.

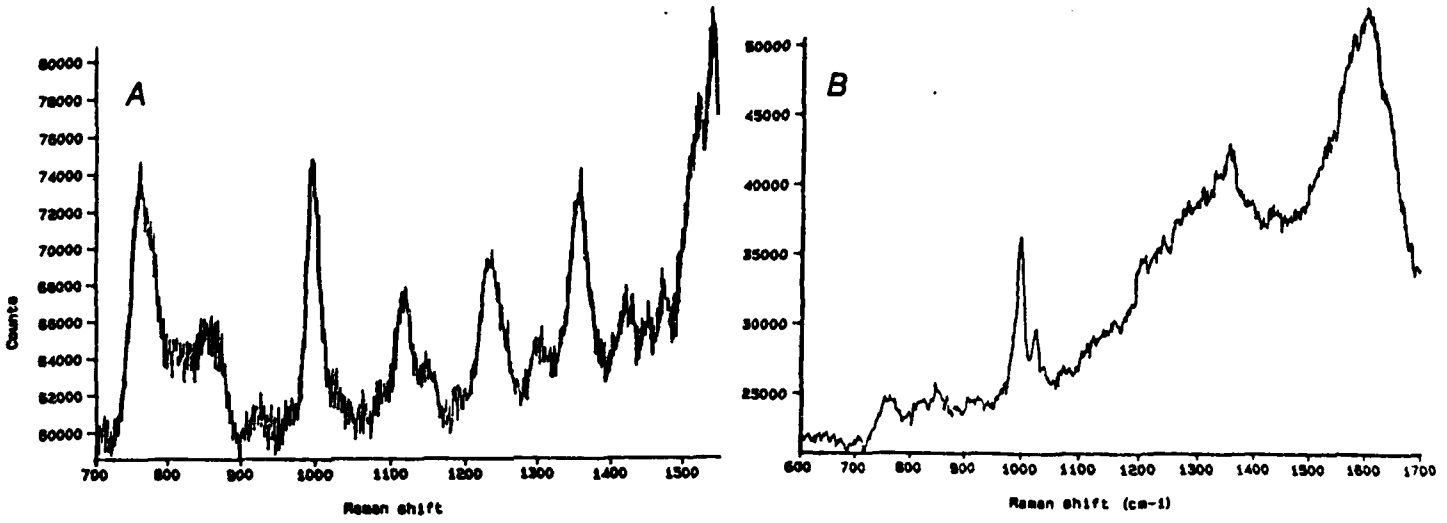

Figure 4. SERS study of aromatic amino acids adsorbed on conditioned Ag electrodes in 0.1 N KCl. A. tryptophan. B. phenylalanine.

Ribulose biphosphate carboxylase (RuBisCo) has been targeted by other ONR Biosurfaces researchers as a putative fouling compounds, so we are performing complementary Raman scattering and EIS studies on this ubiquitous enzyme. In our first attempts, we were unable to obtain acceptable SERS spectra of this enzyme because of fluorescence problems and impurities in the preparation. We are continuing our studies by purifying the enzyme and modifying our sampling techniques so that we may examine non-SERS producing metals as well as SERS-producing metals. Techniques under investigation are: UV Resonance Raman Spectroscopy (pulsed excitation, gated detection), WIRRS, and near IR FT-Raman spectroscopy (instrument expected 3/89).

We have adapted WIRRS techniques as developed by polymer scientists at IBM Research Center to examine thin films adsorbed to substrates, such as glass. In principle, the waveguide provides an extended pathlength for laser excitation light by internal reflection within the thin film. This is accomplished by directing the laser light into the film at a critical grazing angle via a high refractive index prism and the light remains trapped in the film (waveguide) due to mismatch of the refractive indices of the substrate, film, and overlayer (air) (Fig. 5A). Scattered emissions are then collected over the waveguide by means of a lens or linear fiber optic array and focused on the spectrometer slit. Spectra obtained by WIRRS have a much-improved sensitivity and signal-to-noise ratio relative to bulk sample analyses. We have successfully developed a WIRRS apparatus in our laboratory and have obtained excellent Raman spectra of 1 μm films of polystyrene on Pyrex substrata (waveguides courtesy of J. Rabolt, IBM Almaden Research Center, CA) (Fig. 5b). We are in the process learning how to prepare our own waveguides on Pyrex substrata with previously-studied polymers and will then prepare them with putative fouling

molecules. We anticipate being able to apply the same sampling strategy to metal substrata. Our second generation waveguide assembly will employ an environmental chamber to control humidity and temperature thereby permitting examination of hydrated surfaces.

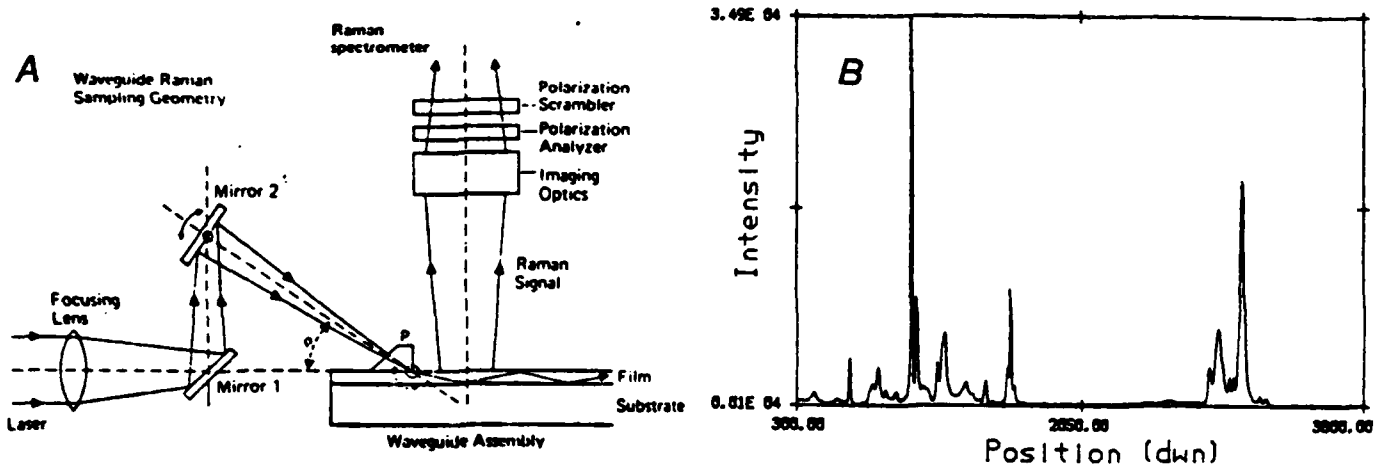

Figure 5. Waveguide Internal Reflectance Spectrometry (WIRRS). A. Schematic of sampling optics. Adjustable mirror 2 and prism (P) determine critical angle of laser beam needed to maximize internal reflectance the film (from Schlotter and Rabolt, 1984). B. Raman spectra of a 1-μm layer of polystyrene adsorbed on Pyrex obtained by WIRRS.

Despite long delays in shipping equipment required for Electrochemical Impedance Spectroscopy (frequency response analyzer, electrochemical interface, and instrumentation controller), B. Liebert and students, M. Nullet, and P. Chong have successfully produced an operational system, including electrochemical cell and operating software. Preliminary runs with a silver electrode, UV-oxidized seawater, and a putative fouling protein, Ribulose 1,5 biphosphate carboxylase (RuBisCo) have been completed.

Prior to adding RuBisCo to the system, a series of runs were made to establish functionality of the cell and system parameters. The system stabilized before each run at approximately -70 mV. A Bode plot of a typical run is indicative of an equivalent circuit consisting of a simple parallel RC circuit in series with an uncompensated resistance (Fig. 6). Although the frequency does not go high enough to determine the uncompensated resistance, it is of the order of 10 ohms or less. There is no indication of any Warburg (diffusional) impedance in this plot as expected since the electrolyte is flowing. A double layer capacitance of 50-100 μF cm^{-2} and a polarization resistance of 250-300 kohm cm^{-2} can be calculated from Fig. 6a.

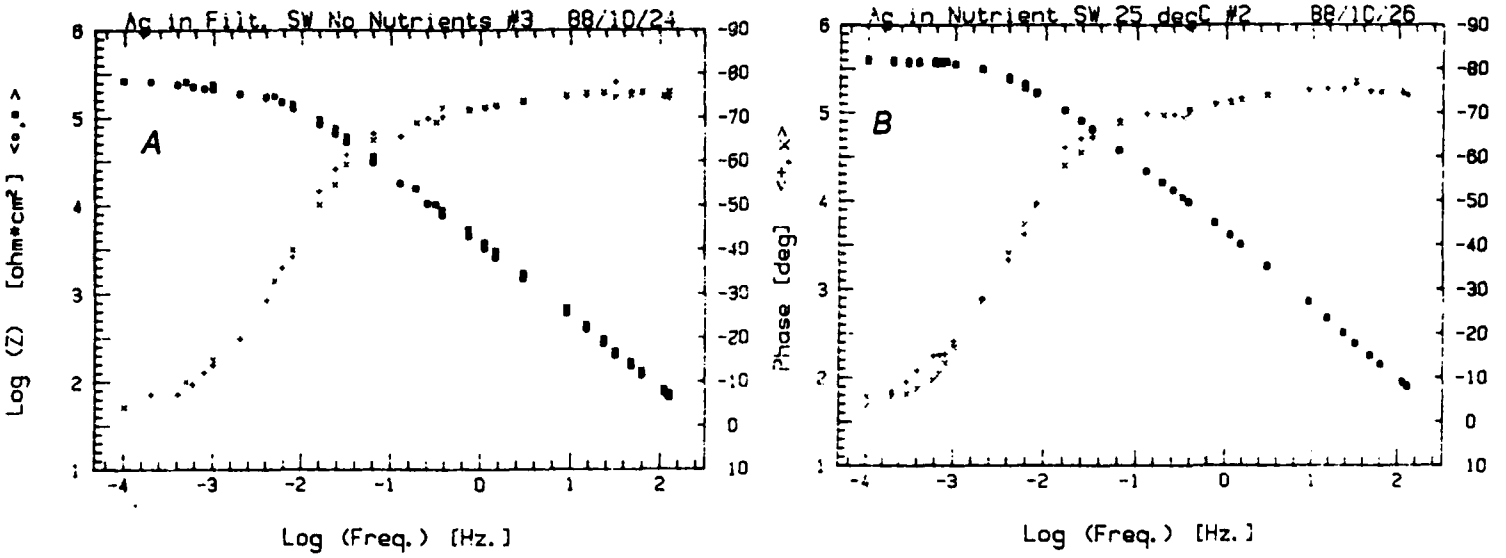

Figure 6. Bode plots for Ag electrode in (A) unamended UV-oxidized seawater, and in (B) UV-oxidized seawater amended with RuBisCo (10 mg l^{-1}).

After amendment with RuBisCo (10 mg l^{-1}; final conc.), the system stabilized at -85 - 100 mV and again indicates a simple equivalent circuit as above (Fig. 6b). After RuBisCo addition, the polarization resistance increased to about 400 kohm cm^{-2}. This is expected as a result of formation of an insulating layer of protein on the working electrode.

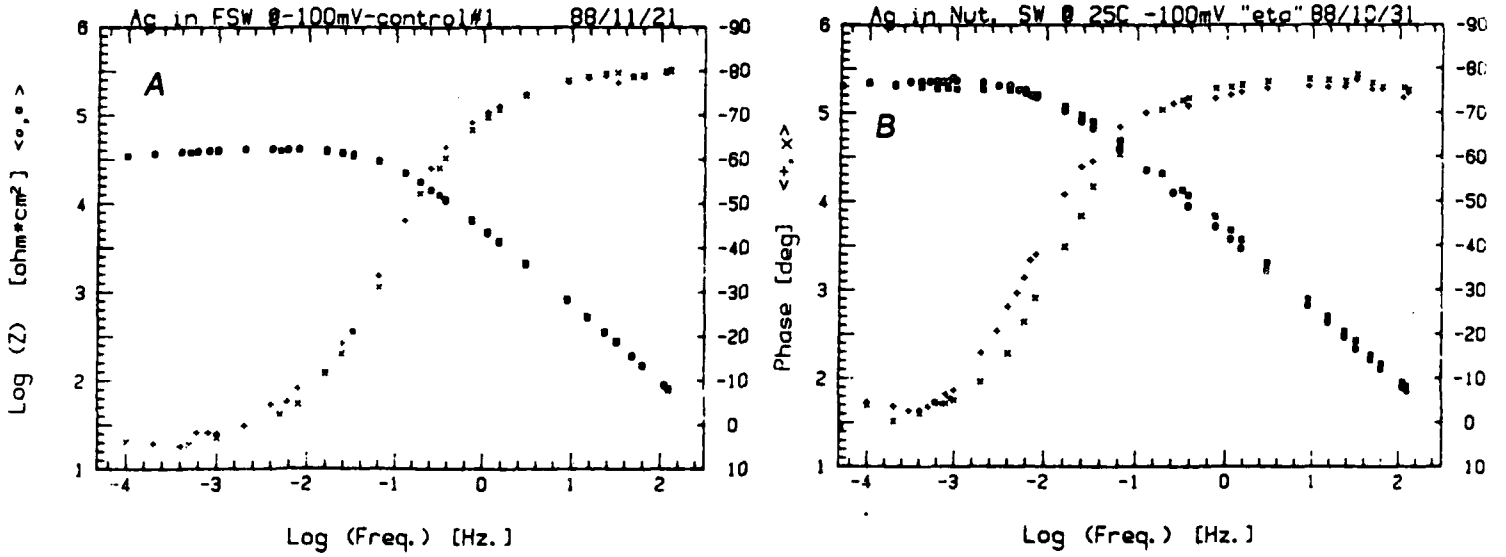

Figure 7. Bode plots for Ag electrode with a -100 mV overpotential applied in (A) unamended UV-oxidized seawater and in (B) UV-oxidized seawater amended with RuBisCo (10 mg l^{-1}).

We ran both systems (unamended and amended seawater) at an overpotential of -100 mV (Fig. 7). Approximately the same equivalent circuit seems to prevail, although the charge-transfer resistance is nearly an order of magnitude larger for the amended seawater sample (Fig. 7b). These results are consistent with the adsorption of the labile polyelectrolytic protein on the surface of the working electrode and the lowering of the potential energy barrier by the application of a -100 mV overpotential.

Our preliminary EIS studies have demonstrated that we can detect changes in the low-frequency impedance caused by the adsorption of a single dissolved solute, a protein, onto a silver electrode immersed in seawater. We have learned the strengths and weaknesses of our experimental system and currently modifications are underway to improve electrode preparation, reproducibility, and extend frequency to 300 kHz. In our continuing studies, we will determine the sensitivity of our system, examine other solutes and other metal coupons before integrating the EIS system into the continuous culture simulation system.

WORK PLAN (YEAR 2):

We are engaged in adapting several non-SERS methods for the examination of films on metals other than silver (WIRRS and UV-RRS). Parallel studies will be performed using FT-IR spectroscopy and EIS. Strategies for desorbing material from coupons will be evaluated by conventional analytical techniques, e.g., HPLC, fluorometry, electrophorhesis, etc. Optimal sampling techniques will be obtained for several classes of fouling compounds under controlled conditions. We are currently producing our own humic acids from phytoplankton cultures which we will refine, molecular size fractionate, characterize spectrophotometrically, and use in kinetic fouling experiments with our Raman probes. In addition to our laboratory simulations, short deployments of test coupons will also be performed in nearshore and offshore environments for comparative purposes as time permits.

INVENTIONS: none

PUBLICATIONS:

G. T. Taylor and S. K. Sharma. 1988. Use of laser spectroscopic probes for examination of biofouling. Presented at the National Institute of Oceanography, Goa, India, Nov. 1988.

TRAINING ACTIVITIES:

Our project currently employs one graduate student and two undergraduates. Our graduate student, Paul Troy, will derive his Ph.D. dissertation from this project (topic presently undeclared) and is receiving training in vibrational spectroscopy, marine

chemistry, and microbiology. Our undergraduates, Eamonn O'Toole and Patty Fisher, are Biology majors and are receiving firsthand laboratory experience in chemistry and microbiology. Mr. Michael Nullet, an M.S. student in Mechanical Engineering, is collaborating with B. Liebert to refine and operate the EIS system. P. Chong, an undergraduate in Electrical Engineering has been instrumental in developing the software necessary for EIS data acquisition and reduction. We have an M.S. student in Microbiology who will begin a directed research project this Spring examining the microbial ecology of our fouled coupons using light and electron microscopy. A Ph.D. student in Botany, Tina Michaels, interested in biofouling of natural and artificial substrata will be collaborating with us starting this Spring. Ms. Michaels already has experience with field deployments of test coupons, SEM, and FT-IR and is the recipient of a fellowship from Hawaii Natural Energy Institute for biofouling work.

AWARDS / FELLOWSHIPS: none